

Title: DAPI Staining of CHO-K1 Cells

Approvals:

Preparer: _____ Date _____
Reviewer: _____ Date _____
Reviewer: _____ Date _____

1. Purpose: Prepare the reagents and stain CHO-K1 cells with the fluorescent nuclear DAPI.
2. Scope: This SOP can be used to stain cells that have been transfected or affinity labeled with multiple, but different colored, fluorescent molecules, or as a single stain if desired.
3. Responsibilities:
 - 3.1. It is the responsibility of the course instructor /supervisor to ensure that this SOP is performed as directed and to update the procedure when necessary.
 - 3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. References:
 - 4.1. SOP: DAPI-01
5. Definitions:
 - 5.1. DAPI = 4',6-diamidino-2-phenylindole
 - 5.2. Excitation Wavelength: In order to activate a fluorescent molecule it must receive high levels of energy to "excite" orbital electrons. The many different fluorescent molecules available often require different wavelengths of energy to achieve this excitation. The specific wavelength for a fluorescent molecule is known as its Excitation Wavelength, For DAPI the Excitation Wavelength is 358nm, which can be generated with a xenon or mercury-arc lamp.
 - 5.3. Emission Maximum: After the electrons are excited" they often change orbital levels where they are sustained for a short period. Unable to continue to hold the extra energy, it is released by the electrons as they return to their original orbits. The released energy is detected as a wavelength of some color (commonly, red, yellow, green or blue). The specific wavelength, and therefore color, is called the Emission Maximum. For DAPI the Emission Maximum is 461 nm, which is in the blue color range of the visible light spectrum.
6. Precautions:
 - 6.1. DAPI is a known mutagen and must be handled with care while wearing full PPE gear.
 - 6.2. Dispose of waste dye safely and in accordance with applicable local regulations.

7. Materials:

- 7.1. DAPI Hydrochloride (MW = 350.3), 5 mg/mL stock (14.3 mM)
- 7.2. Phosphate-buffered saline
- 7.3. CHO-K1 cells (~80% confluence)
- 7.4. fluorescent microscope with appropriate filter set

8. Procedure:

8.1 General

- 8.1.1. Obtain the desired cells for nuclear staining.

8.2. Prepare DAPI stock solution following SOP: DAPI-01

- 8.2.1. Dissolve 10 mg of DAPI Dihydrochloride in 2 mL of deionized H₂O

NOTE 1: Only moderately soluble in H₂O, full dissolving may take some time.
Sonication generally will help

NOTE 2: dimethylformamide (DMF) may be used in place of H₂O with better success

NOTE 3: For long term storage, aliquot and store at -20°C in foil. For short term storage solution may be kept at 2° – 6°C. Generally stable for 6 months.

8.3. Prepare DAPI working solution (300 nM)

- 8.3.1. Mix 1 uL (microliter) of DAPI stock solution (14.3 mM) with 50 mL (milliliter) of PBS.

- 8.3.2. Cover with foil and store at 2° – 6°C until use.

NOTE 4: Aliquot remaining dye and store at -20°C for future use.

8.4. Prepare cells for staining

NOTE 5: Cells may be prepared in a variety of ways for nuclear staining;
washed, unfixed cells
fixed, unpermeabilized cells
fixed, pre-labeled with affinity molecules
fixed, transfected cells with fluorescence associated with cellular structures, such as we will be using

- 8.4.1. Obtain a sample of the cells we transfected and fixed earlier. These will have a variety of single and dual fluorescent labels in the form of GFP-actin and/or RFP-Mitochondria.

- 8.4.2. Wash the cells with PBS.

- 8.4.3. Equilibrate the cells by leaving them covered with PBS for ~3 minutes

- 8.4.4. Remove and discard the PBS.

- 8.4.5. Add enough DAPI working solution to cover the cells completely

8.4.6. Incubate at room temperature for ~3 – 5 minutes.

8.4.7. Rinse the cells several times with PBS.

8.4.8. After the last rinse, the cells may be viewed under the appropriate conditions using a fluorescent microscope equipped with the proper filters.

NOTE 6: If the microscope is properly equipped, we should be able to overlay the various fluorescent images to obtain a 3 color micrograph of our cells in green, red and blue.

9. Attachments:

9.1. None

10. History:

Name	Date	Amendment
William H Woodruff	15May2014	Initial release